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(54) Title: PROCESS FOR CHARACTERISING NUCLEIC ACIDS IN SOLUTION

(57) Abstract: The invention provides a method for ascertaining information relating to nucleic acid material in a solution, comprising: measuring a parameter relating to the conductivity of the solution at a multiplicity of temperatures; analysing the measurements of said parameter; and detecting a discontinuity in the change of said parameter with temperature.

Process for characterising nucleic acids in solution

The invention relates to processes for characterisation of nucleic acid and to apparatuses for use in such processes.

Many molecular processes require an end point characterisation of nucleic acids in order to determine process outcome and/or efficiency. Conventionally, nucleic acids are characterised by a process of gel electrophoresis, which separates the nucleic acids according to their overall charge and molecular weight. The nucleic acid is subsequently visualised using fluorescent dyes that intercalate with the double stranded DNA. Those dyes are generally highly toxic and many are carcinogenic. The distance the DNA migrates through the gel matrix is measured in order to determine the molecular weight of the nucleic acid. Electrophoretic separation is time-consuming and is a technically involved process, resulting in bottlenecks for many molecular processes including DNA diagnostics. Consequently, the characterisation of DNA forms a major cost in the diagnostic procedure and there is a need for faster and more economical methods of DNA characterisation.

It is known from WO97/32039 that certain characteristics of nucleic acids in solution, for example the concentration and the molecular weight, can be ascertained by measuring the electrical conductivity of the solution. In WO99/10530 it is disclosed that the molecular weight of nucleic acids in solution can be determined from the conductivity of the solution when an alternating current is applied.

It has now been found that useful information regarding the DNA components of a sample may be obtained by examining the variation of the conductivity of the sample with temperature. Conductivity measurements for the purpose of

that examination may be derived from measurements of any parameter of the sample that is related to conductivity.

The invention provides a method for ascertaining information relating to nucleic acid material in a solution, comprising:

measuring a parameter relating to the conductivity of the solution at a multiplicity of temperatures;

analysing the measurements of said parameter; and

detecting a discontinuity in the change of said parameter with temperature.

Preferably, the parameter measured is selected from conductivity, impedance, and resistance. Most preferably, the parameter is conductivity.

The variation with respect to temperature of the conductivity of a solution containing one or more nucleic acids comprises two components: firstly a general linear increase in conductivity with increasing temperature, as is expected for most electrolytes (this will be referred to hereinafter as the background increase in conductivity); and, secondly, a further variation that is characteristic of the nucleic acids in the solution. (This will be referred to hereinafter as the specific variation of conductivity.)

Specific variations of conductivity occur only in certain temperature ranges and there are thus discontinuities in the relationship between bulk conductivity and temperature at particular temperatures.

The temperature at which a discontinuity is observed may be used to diagnose denaturation of a nucleic acid in the solution.

The temperature at which a discontinuity occurs is related to the molecular weight of the relevant nucleic acid present in the solution. Accordingly the temperature at which a discontinuity is observed may be used to form the basis of an estimation of the length of a nucleic acid. The

specific variation may be an increase or a decrease in conductivity.

The absolute magnitude of the change of the rate of change of the parameter with temperature may be used to estimate the quantity of nucleic acid that denatures at that temperature.

The method can be carried out with any nucleic acid. Preferably the nucleic acid is RNA or DNA. Most preferably the nucleic acid is DNA.

The nucleic acids may be the products of a nucleic acid amplification reaction, for example the polymerase chain reaction.

The solution may contain a mixture of nucleic acids, at least two discontinuities being detected and each discontinuity corresponding to a respective nucleic acid component of the solution. The method of the invention may be used for samples comprising nucleic acids and species other than nucleic acids. It may, for example, be used for samples comprising nucleic acids in the presence of ionic species other than nucleic acids.

The invention thus provides an on-line process for characterising nucleic acids in solution, circumventing the need for end-point detection. Unlike the previously known processes, this invention is applicable to nucleic acid profiling in both buffered and salt free solution. It is therefore suited to a wide range of molecular reactions where nucleic acids are modified either chemically or biochemically to produce products of various molecular weights, for example monitoring changes in the distribution of nucleic acid species during and/or at the end of the polymerase chain reaction (PCR). The process can also be utilised in applications where the user wishes to distinguish between double and single stranded DNA; for example for homogenous hybridisation experiments. The

process can be utilised in any application where the user wishes to detect a hybridisation or a denaturation event. Such events include hybridisation between or denaturation of nucleic acids of different lengths and hybridisation between or denaturation of nucleic acids that are not complementary along the whole of their lengths, as may be the case, for example, in a hybridisation array.

The invention makes use of bulk conductivity changes which are believed to occur during the thermal melting of nucleic acids in solution. This is achieved by measuring the increase in the bulk conductivity of the nucleic acid solution resulting from strand separation at defined temperatures where the double stranded nucleic acid separates into single stranded nucleic acid (melting). This temperature and associated changes in bulk conductivity are directly related to the molecular weight of any double stranded nucleic acid present in the solution. Unlike conventional measurements, nucleic profiling can be carried out in the presence of other charged molecules such as ionic buffers, charged nucleotides and contaminating ionic salts.

"Molecular weight" is understood in the context of nucleic acids to refer to the length of a nucleotide in numbers of bases. Accordingly, "molecular weight", "strand length", "chain length" and "length" may be used interchangeably in this context.

The polyelectrolytic behaviour of DNA stems from interaction between the negatively charged phosphate groups of the sugar-phosphate backbone of the double helix, and positively charged counterions. These counterions have been shown to interact with the hydration shell of oligonucleotide duplexes. Specifically, Mg^{2+} recognises the sequence of DNA (through its overall hydration state) by forming outer-sphere complexes with oligomers containing exclusively dA-dT base pairs and inner-sphere complexes with

dG.dC oligomers. (Buckin *et al*, "Mg^{2+} recognises the sequence of DNA through its hydration shell", *J. Am. Chem. Soc.*, 1994, **116**, 9423-9429)

Consequently, a nucleic acid solution can be regarded as a series of electrically distinct phases (a) the double helix, (b) the positively charged ion cloud surrounding the nucleotide molecule, and (c) the aqueous electrolyte (Bone and Small, "Dielectric studies of ion fluctuation and chain bending in native DNA", *Biochemica and Biophysica Acta*, 1995, **1260**, 85-93). The weakly polar nucleotide core of radius 10Å exhibits a low conductance and permittivity in contrast to the relatively high conductance and permittivity of the ionic layer. This ionic phase comprises counterions that are strongly associated with the regularly spaced (1.7Å for ß-DNA) ionic sugar-phosphate groups and a more diffuse ionic layer that is influenced by the static phosphate charge at least 7Å into the aqueous electrolyte.

Thermal denaturation involves a lowering of the bulk hydrate and counterion concentration surrounding the double helix. Removal of these stabilising agents increases the electrostatic repulsion of the negative phophodiester groups resulting in the instability of the double helix configuration. As a consequence, there is an increase in free counterion concentration in the bulk solution. We have found that by measuring the release of these counterions from the DNA hydration layer during thermal denaturation through changes in the conductivity of the solution, it is possible to determine the size distribution and concentration of nucleic acids in that solution.

Interestingly, these counterions also contribute to the anisotropic ion flow across the DNA molecule. This is thought to produce an asymmetric ion atmosphere around the molecule, resulting in an orientating torque on the DNA

molecule. The resulting flow of ions and the resulting torque generated will determine what contribution the DNA molecule makes to the observed conductivity. The conductivity response of nucleic acids to temperature is therefore likely to be a complicated association between the availability of counterions contributing to the bulk conductivity of the solution, and differences in the apparent dipole moments of single and double stranded DNA molecules.

This invention further provides the characterisation of nucleic acid species in solution by a process of thermal denaturation and simultaneous conductimetric and/or impedametric analysis of the nucleic acid solution. Changes in the impedance measurements of the solution can also be monitored and these reflect changes in the distribution of capacitive and ionic components. The process described can be used to identify and characterise different nucleic acids or mixtures of nucleic acids in a mixed population.

Apparatus

Bulk conductivity of a solution of interest is measured using a micro electrode.

The preferred sensor consists of a working electrode and a counter electrode manufactured as an interdigitated array on a suitable substrate such as silicon, glass or polycarbonate. Reference electrodes may be used but are not necessary. The electrodes may be of any suitable material. Inert metals such as platinum, gold and silver, carbon, graphite, carbon-pastes and platinum inks, modified electrodes where electron transfer is mediated by electron-accepting or electron-donating compounds may also be used. Electrode geometry may include any convenient symmetry. Spherical, hemispherical, disk-shaped, plate-shaped, ring-shaped and linear electrodes which form single thin wire electrodes, screen-printed, interdigitated or multiple

arrays of sensing units may be used. Electrodes may be of macro, micro or ultra-micro dimensions. Geometries that maximise the sensitivity of the conductivity measurement are preferred.

In a preferred embodiment, the micro electrodes are constructed from PTFE (Teflon (RTM)) coated silver wire of 0.25mm diameter with 1mm of silver exposed. In use, the microelectrode assembly is immersed in the DNA solution such that the exposed sensing element is completely submerged. By modulating an applied electronic signal between the working electrode and a counter electrode it is possible to determine the distribution of nucleic acid molecules in the sample by analysing the resulting changes in conductivity as the temperature is increased.

The conductivity of the solution is measured using a standard conductivity meter. The applied a.c. voltage may have a frequency of from 1 to 100000Hz, preferably from 10 to 10000 Hz, typically 1000 Hz. The applied voltage may be from 0.1mV to 100V, preferably from 10mV to 1V peak to peak. Alternatively, a d.c. voltage may be used.

The output reading from the conductivity meter is preferably passed through an analogue digital converter to a computing means.

The temperature of the solution of interest is varied by a heating and/or a cooling means. Typically, the temperature of the solution is increased from about 30°C up to about 95°C at a defined ramp rate. The temperature is measured and it is optionally also passed to the computing means. Accurate control and measurement of the temperature improve the sensitivity of the method.

The Method

The change in bulk conductivity of a nucleic acid-containing solution with temperature is measured. Various rates and regimes of temperature variation may be used. An

increase of temperature with time or a decrease of temperature with time may be used. Generally an increase of temperature with time is used. The rate of temperature variation may range from 0.1 to 50ºC per second and it may be linear or non-linear, and continuous or stepped. A correspondingly high rate of data (conductance and temperature measurement data) acquisition is required for high rates of temperature variation. Typically between 0.2 and 20 bulk conductivity measurement data points are recorded per ºC variation in temperature. Preferably between 0.5 and 5 data points are recorded per ºC variation in temperature. It is found that there is a change in conductivity of nucleic acid solutions in addition to the normal background increase associated with an increase in temperature of the solution alone. The change in this gradient at specific temperatures (the specific variations) is indicative of the melting temperature of the specific nucleic acid species in the test solution. Low molecular weight molecules are associated with a change in conductivity gradient at lower temperatures than high molecular weight nucleic acid molecules. In addition, multiple changes in conductivity gradient during heating are diagnostic of multiple nucleic acid species in solution.

The temperature at which a discontinuity in conductivity occurs is determined by analysing the conductivity vs temperature data-set for departures from continuous approximately linear behaviour. This analysis may be carried out by linear regression analysis or any other suitable means.

From the melting temperature of a nucleic acid (found as the temperature at which the discontinuity in conductivity occurs) it is possible to estimate the molecular weight and the oligomer chain length of the nucleic acid. This may be done using known formulae

(calculated according to, for example, Breslauer, K. J., *Proc. Natl. Acad. Sci.*, 1986, 3746-3750) or tables. Known formulae or tables relate melting temperature to oligomer chain length and may take into account factors such as base composition (i.e. C/G : A/T ratio), ionic strength and the presence of other molecules, for example formamide. Alternatively, it may be done by calibrating the apparatus and solution conditions using oligomers of known chain length.

The specific variation may be an increase or a decrease in addition to the back-ground increase in conductivity. The absolute magnitude of the specific variation is proportional to the quantity of the relevant nucleic acid present. Accordingly, the relevant nucleic acid may be quantified using the method of the invention.

We have found that the molecular weight distribution for solutions containing single or multiple nucleic acid species can be analysed by monitoring the temperature at which there is a change in the gradient of the bulk conductivity of the solution in addition to the normal background increase in conductivity associated with the increase in temperature. Moreover, we have found that it is possible to measure these changes above a background of other contaminating salts and charged molecules, that enables this process to be used to determine the distribution of products from a molecular reaction, for example the polymerase chain reaction.

Applications

This invention provides an integrated method for characterising nucleic acid material in solution. It can be used in any process where the operator wishes to analyse the distribution of nucleic acids in a buffered or salt free sample, or to distinguish between single and double stranded nucleic acid species in a sample. The process can also be

used as a diagnostic tool for changes in the distribution of nucleic acid material during molecular reactions, for example the Polymerase Chain Reaction.

The Polymerase Chain Reaction (PCR)

US Patent No. 4,683,195 (Cetus Corporation) discloses a process for amplification of nucleic acid by the polymerase chain reaction (PCR). Short oligonucleotide sequences usually 10-40 base pairs long are designed complementary to flanking regions either side of the target sequences to be amplified. These primers are added in excess to the target sequence DNA. A suitable buffer, magnesium chloride ions, a thermostable polymerase and free nucleotides are also added. A process of thermal cycling is typically used to amplify the DNA typically several million-fold. The target DNA is initially denatured at 95ºC and then cooled to generally between 40ºC to 60ºC to enable annealing of the primers to the separated strands. The temperature is then raised to the optimal temperature of the polymerase, generally 72ºC, which then extends the primer to copy the target sequence. This series of events is repeated (usually 20 to 40 times). During the first few cycles, copies are made of the target sequence. During subsequent cycles, copies are made from copies, increasing target amplification exponentially. The use of thermal cycling has a number of disadvantages. It requires the use of thermostable enzymes that preclude the use of more efficient polymerases that are generally heat labile. It is dependent on a process of temperature control that is inherently slow, is broadly unreliable and does not lend itself to processing large numbers through process miniaturisation. Many thermostable polymerases have low-fidelity that consequently results in high rates of misincorporation. Analysis of results requires further manipulation of the sample and is time consuming.

This invention has direct application to monitoring the PCR, specifically the characterisation of nucleic acid material being amplified during the reaction process and at the end or during the reaction. This has the advantage of removing the requirement for further end point sample processing, reducing the time taken in performing the PCR assay. In addition, it allows the user to monitor the amplification of multiple amplicons in real time and to make intelligent decisions about the performance of a reaction, for example to alter the specificity of a reaction, in real time.

For the monitoring of a PCR process the cycling of temperature during the PCR cycle may be used for the temperature variation during the method. This has the advantage that no sample needs to be taken out of the reaction vessel. Alternatively, an aliquot may be removed from the reaction. The presence of magnesium (as in most PCR protocols) reduces nucleic acid melting temperatures so calibration experiments may be required in order to obtain accurate assessment of nucleic acid weights.

This detection process also allows qualitative and quantitative information to be extracted from the reaction. As a qualitative tool, it gives information about the distribution of products in a reaction, telling the operator whether single or multiple products have been amplified. In addition, it can be used to provide information about the size distribution of products at the reaction. Since specific changes in the rate of change of conductivity with temperature measured during thermal denaturation are proportional to the number of ionic molecules (e.g. Mg^{2+}) released from the nucleic acid molecule, it follows that the magnitude of the change in gradient is related to the molecular weight of and is proportional to the quantity of the specific nucleic acid molecule present in solution.

This process can be used to simultaneously determine the distribution (molecular weight and concentration) of molecules being generated during a PCR. Unlike conventional methods, this process can be used in real time at each cycle to generate a profile of amplicon synthesis at different stages of a PCR. Generally amplicons are generated at different stages of a reaction. For example, primer dimer artefacts are generally only synthesised during late stages of a reaction. The user can use the present invention to terminate a diagnostics reaction early once a product of the correct size has been sythesised, and critically before the production of PCR artefacts such as primer dimers which are normally synthesised during the later cycles of the reaction. This improves the process since it allows the user to make intelligent choices about terminating or modifying reactions on-line to limit or remove false positives. In addition, obtaining information about the performance of a reaction in real time can be used to alter the cycling conditions in order to promote the synthesis of a specific amplicon.

The invention further provides an apparatus comprising

- a means for heating a sample comprising nucleic acid;
- a means for detecting a parameter of the sample related to conductivity; and
- a means for detecting a temperature at which there is a discontinuity in the change in the parameter with temperature.

Brief description of the figures:

Fig. 1 The conductimetric melting curve for a PCR that generates a single high molecular weight amplicon (800bp) (Example 1);

Fig. 2 The conductimetric melting curve for a PCR that generates a high molecular weight

amplicon together with primer dimer artefacts (Example 2);

Fig. 3 The conductimetric melting curve for a PCR generating a low molecular weight amplicon (450bp) together with primer dimer artefacts (Example 3); and

Fig. 4 The conductimetric melting curve for four products of different molecular weights derived from a PCR reaction (Example 4).

The following Examples further illustrate the invention:

Example 1

A standard PCR amplification of an 800 base pair DNA fragment from Salmonella target DNA was carried out using an MJ Thermal cycler. The conductivity of the solution was measured using a Jenway conductivity meter connected to a PC via an analogue digital converter (Computer Boards PCL 812 Pts) for data acquisition. The conductimetric melting curve for this PCR after a suitable number of cycles generating a single, high molecular weight amplicon is shown in Figure 1. It is seen in the figure that the conductivity increases in a linear manner with temperature at low temperatures (the line having a formula $y = 0.0017x + 0.5267$ with $R^2 = 0.9987$, where y is the bulk conductivity in mS/cm and x is the temperature in °C). A discontinuity occurs at approximately 74°C, which temperature corresponds to the melting temperature for DNA of 800 bp length in a salt buffered solution. Beyond the discontinuity point, the conductivity again increases in a linear manner with temperature but with a greater gradient ($y = 0.0021x + 0.5011$ with $R^2 = 0.9979$).

Example 2

Using the same apparatus, materials, primers and target DNA as in Example 1, a PCR reaction was carried out. After multiple cycles, the reaction gave rise to significant primer dimer artefacts in addition to the 800bp amplicon. The conductimetric melting curve for this PCR after a suitable number of cycles is shown in Figure 2. It is seen in the figure that the conductivity increases in a linear manner with temperature at low temperatures (the line having a formula $y = 0.0019x + 0.514$ with $R^2 = 0.9945$). A discontinuity occurs at approximately 49°C, which temperature corresponds to the melting temperature of low molecular weight primer dimer artefacts in a salt buffered solution. Above 49°C, the conductivity of the solution increases in a linear manner with temperature (the line having a formula $y = 0.0026x + 0.479$ with $R^2 = 0.9981$) until a second discontinuity occurs at approximately 70°C. The second discontinuity occurs at a temperature corresponding to the melting temperature of the 800bp amplicon. Beyond the discontinuity the conductivity of the solution increases in a linear manner with temperature (the line having a formula $y = 0.0020x + 0.520$ with $R^2 = 0.9842$).

A further discontinuity at approximately 78°C is seen at which point the curve becomes a line of formula $y = 0.0013x + 0.5776$ with $R^2 = 0.9923$ as a result of the effects of the nucleic acids being dominated by the background increase in conductivity of the solution with temperature at high temperatures.

Example 3

Using the same apparatus as in Example 1, a 450bp fragment of human target DNA was amplified. The conductimetric melting curve for this PCR after a suitable

number of cycles is shown in Figure 3. It is seen in the figure that the conductivity increases in a linear manner with temperature at low temperatures (the line having a formula $y = 0.0020x + 0.4506$ with $R^2 = 0.9917$). A discontinuity occurs at approximately 47°C, which temperature corresponds to the melting temperature of low molecular weight primer dimer artefacts in a salt buffered solution. Above 47°C, the conductivity of the solution increases in a linear manner with temperature (the line having a formula $y = 0.0032x + 0.3951$ with $R^2 = 0.9984$) until a second discontinuity occurs at approximately 53°C. The second discontinuity occurs at a temperature corresponding to the melting temperature of the 450bp amplicon. Beyond the discontinuity the conductivity of the solution increases in a linear manner with temperature (the line having a formula $y = 0.0025x + 0.4376$ with $R^2 = 0.9969$).

A further discontinuity at approximately 78°C is seen at which point the curve becomes a line of formula $y = 0.0020x + 0.4741$ with $R^2 = 0.996$ as a result of the effects of the nucleic acids being dominated by the background increase in conductivity of the solution with temperature at high temperatures.

Both primer dimer and amplicon discontinuities are visible on the graph. The second discontinuity point for the 450bp product occurs at a lower temperature than that observed for the 800bp product in Examples 1 and 2.

Example 4

A PCR reaction was carried out that gave rise to four products of different molecular weight. The conductimetric melting curve is shown in Figure 4. It is seen in the figure that the conductivity increases in a linear manner with temperature at low temperatures (the line having a

formula $y = 0.0019x + 0.5267$ with $R^2 = 0.9942$). A discontinuity occurs at approximately 43°C, which temperature corresponds to the melting temperature of the lowest molecular weight amplicon in the salt buffered solution. Above 43°C, the conductivity of the solution increases in a linear manner with temperature (the line having a formula $y = 0.0023x + 0.5092$ with $R^2 = 0.9976$) until a second discontinuity occurs at approximately 63°C. The second discontinuity occurs at a temperature corresponding to the melting temperature of the second lowest molecular weight amplicon. Beyond the second discontinuity the conductivity of the solution increases in a linear manner with temperature (the line having a formula $y = 0.0018x + 0.5422$ with $R^2 = 0.9912$) until a third discontinuity occurs at approximately 72°C. The third discontinuity occurs at a temperature corresponding to the melting temperature of the second highest molecular weight amplicon. Beyond the second discontinuity the conductivity of the solution increases in a linear manner with temperature (the line having a formula $y = 0.0015x + 0.5597$ with $R^2 = 0.9916$) until a fouth discontinuity occurs at approximately 87°C. The fourth discontinuity occurs at a temperature corresponding to the melting temperature of the highest molecular weight amplicon. Beyond the fourth discontinuity the conductivity of the solution increases in a linear manner with temperature (the line having a formula $y = 0.0014x + 0.5702$ with $R^2 = 0.9700$). Four products of different molecular weight are clearly distinguishable. Each product is correlated to a change in conductivity rate at a temperature indicative of its molecular weight.

A further discontinuity at approximately 87°C is seen at which point the curve becomes a line of formula $y = 0.00110x + 0.5943$ with $R^2 = 0.9744$ as a result of the effects of the

nucleic acids being dominated by the background increase in conductivity of the solution with temperature at high temperatures.

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Claims

1. A method for ascertaining information relating to nucleic acid material in a solution, comprising:

measuring a parameter relating to the conductivity of the solution at a multiplicity of temperatures;

analysing the measurements of said parameter; and

detecting a discontinuity in the change of said parameter with temperature.

2. A method according to claim 1, in which the parameter is conductance.

3. A method according to claim 1, in which the parameter is impedance.

4. A method according to any one of claims 1 to 3, in which a temperature at which a discontinuity is observed is used to diagnose denaturation of a nucleic acid in the solution.

5. A method according to any one of claims 1 to 4, in which a temperature at which a discontinuity is observed is used to form the basis of an estimation of the length of a nucleic acid.

6. A method according to any one of claims 1 to 5, in which the magnitude of the change of the rate of the change in parameter with respect to temperature is used to estimate the quantity of the nucleic acid present that denatures at that temperature.

7. A method according to any one of claims 1 to 6, wherein the nucleic acid is DNA or RNA.

8. A method according to any one of claims 1 to 7, wherein the nucleic acid is DNA.

9. A method according to any one of claims 1 to 8, wherein the nucleic acid is produced in a nucleic acid amplification reaction.

10. A method according to claim 9, wherein the nucleic acid is DNA and wherein the DNA is produced in a polymerase chain reaction.

11. A method according to any one of claims 1 to 10, wherein the solution contains a mixture of nucleic acids and at least two discontinuities are detected, each discontinuity corresponding to a respective nucleic acid component of the solution.

12. A method according to any one of claims 1 to 11, wherein the solution comprises nucleic acids and species other than nucleic acids.

13. A method according to claim 12, wherein the solution comprises nucleic acids and ionic species other than nucleic acids.

14. A method according to any one of claims 1 to 13, in which the parameter is measured at a multiplicity of temperatures in the range of from 30ºC to 95ºC.

15. A method according to any one of claims 1 to 14, in which the temperature is increased continuously.

16. A method according to any one of claims 1 to 15, in which the temperature is increased stepwise.

17. A method according to claim 15 or claim 16, in which at least in a portion of the range of temperature through which the solution is heated, from 0.2 to 20 conductance measurements are recorded per ºC change in temperature.

18. A method of characterising nucleic acid material substantially as described in any of Examples 1 to 4 herein.

19. A computer program product so arranged as to cause a computer to implement a method as claimed in any one of claims 1 to 18.

20. A computer program product which causes a computer so to operate:

that it takes as an input a data set signal representing a measurement of a parameter relating to the conductivity of a solution at a multiplicity of temperatures;

that it detects a discontinuity in the change of said parameter with temperature; and

that it converts the data set signals into an output signal that represents a temperature at which a discontinuity in the change of conductivity with temperature takes place.

21. Use of the method of any one of claims 1 to 18 for assessing the composition of a sample of nucleic acid.

22. Use according to claim 21, wherein the chain lengths of the one or more nucleic acids are assessed.

23. Use according to claim 21, wherein the quantity of one or more nucleic acids of various chain lengths is assessed.

24. Use according to any one of claims 21 to 23, wherein the chain lengths of the one or more nucleic acids and the quantity of the one or more nucleic acids of various chain lengths are assessed.

25. An apparatus comprising

- a means for heating a sample comprising nucleic acid;
- a means for detecting a parameter of the sample related to conductivity; and
- a means for detecting a temperature at which there is a discontinuity in the change in the parameter with temperature.

26. An apparatus according to claim 25, comprising means for carrying out a polymerase chain reaction on the sample.

27. An apparatus according to claim 25 or claim 26, in which a plurality of samples may be analysed.

Fig.1.

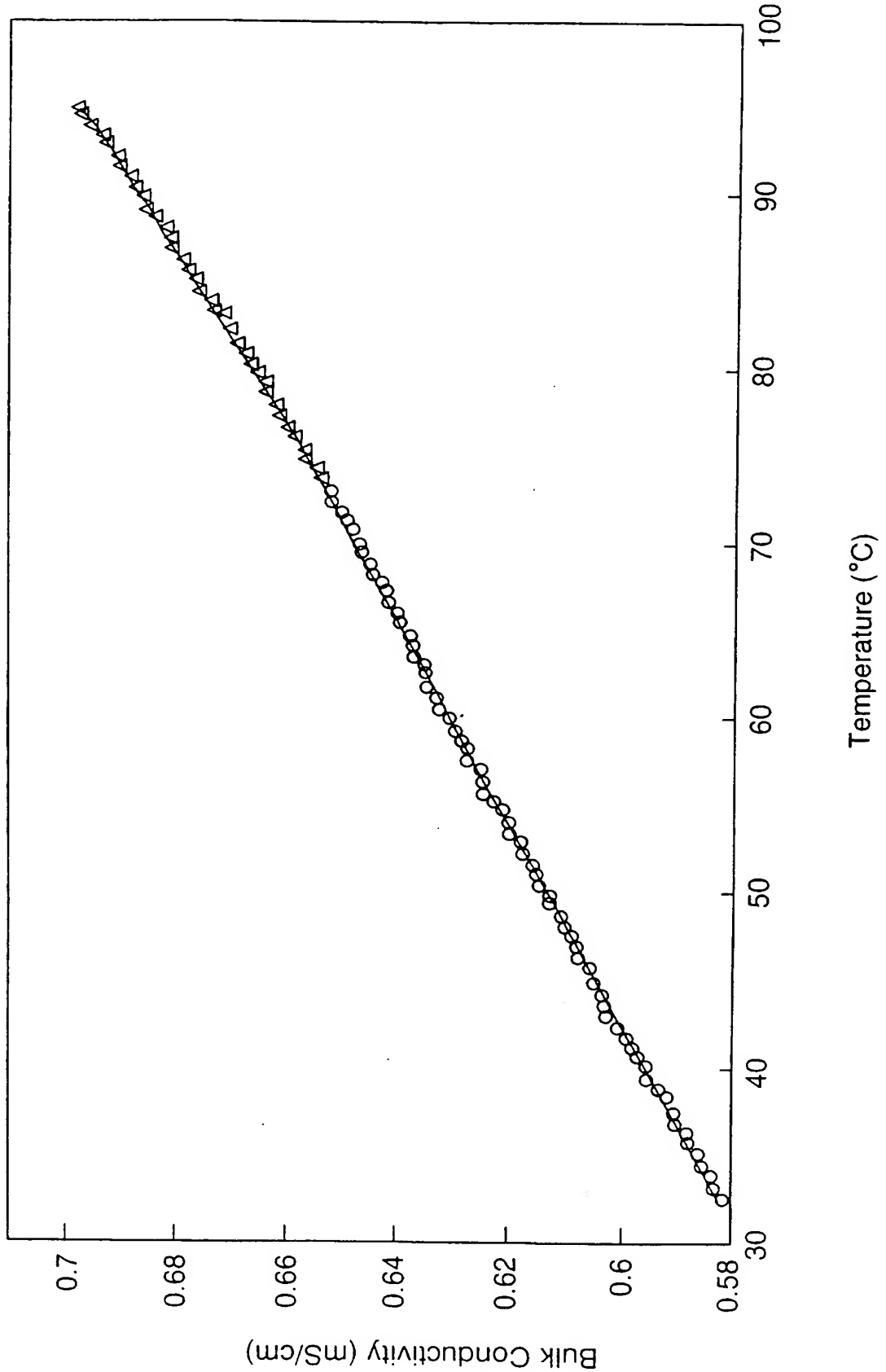


Fig .2.

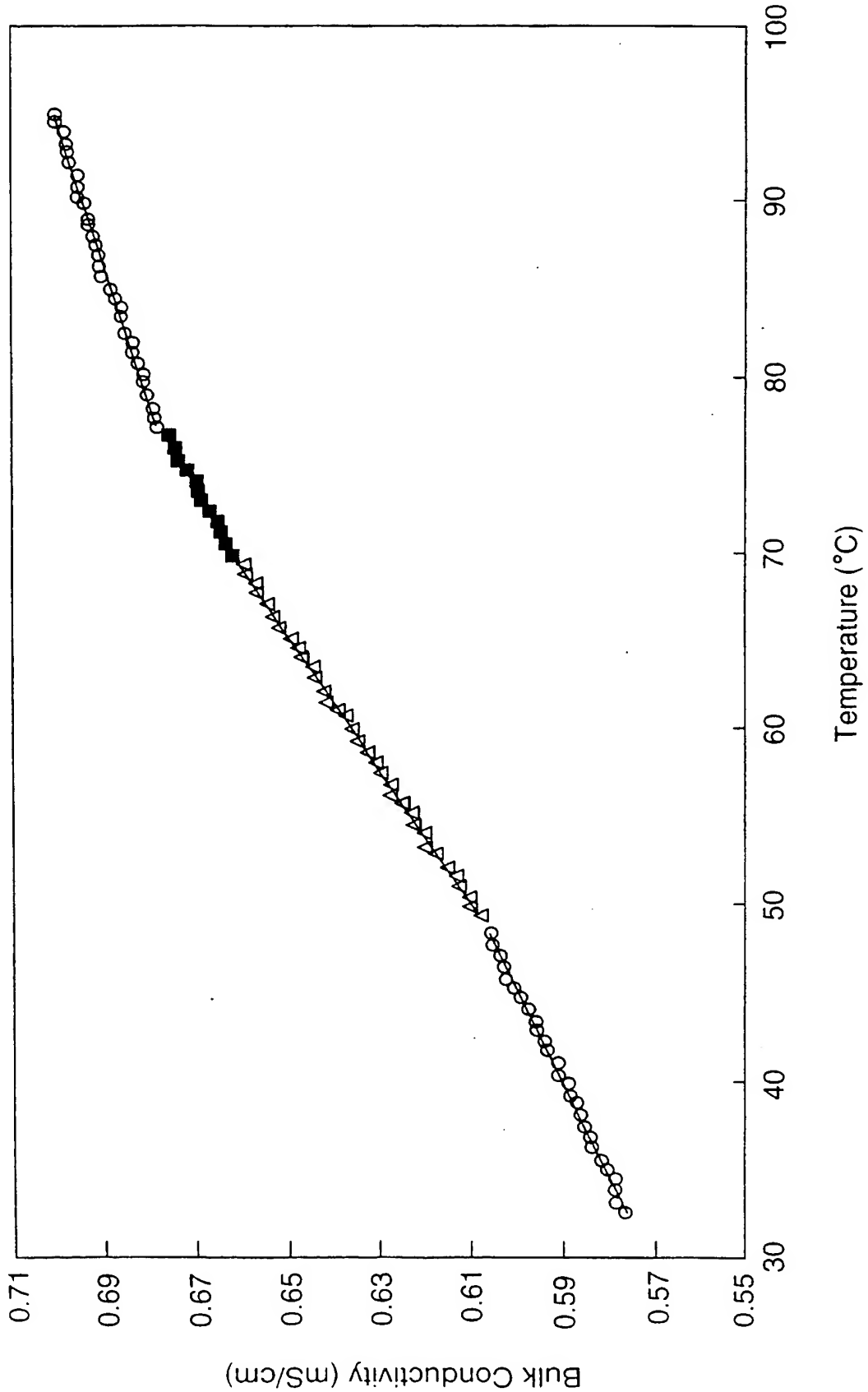
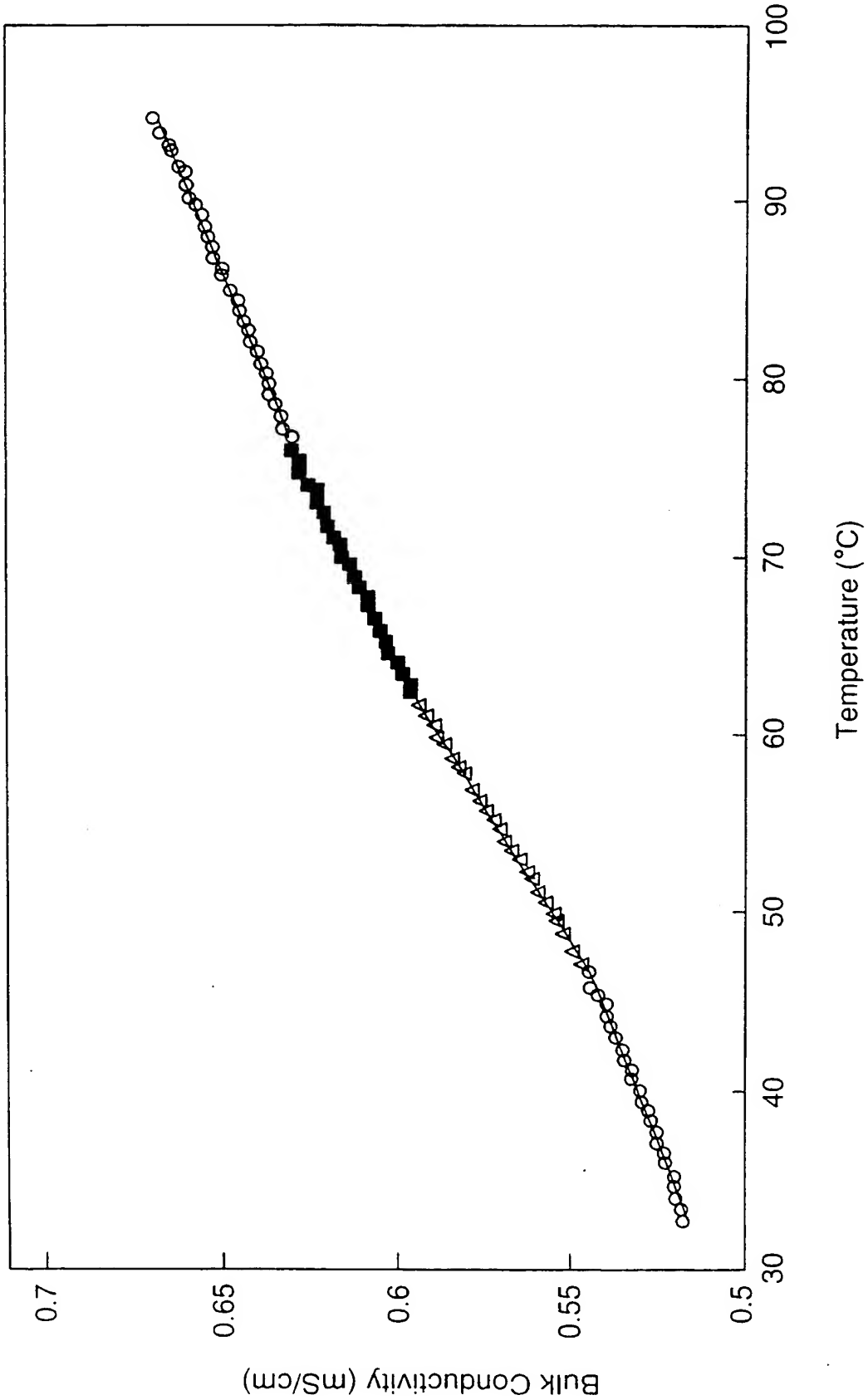
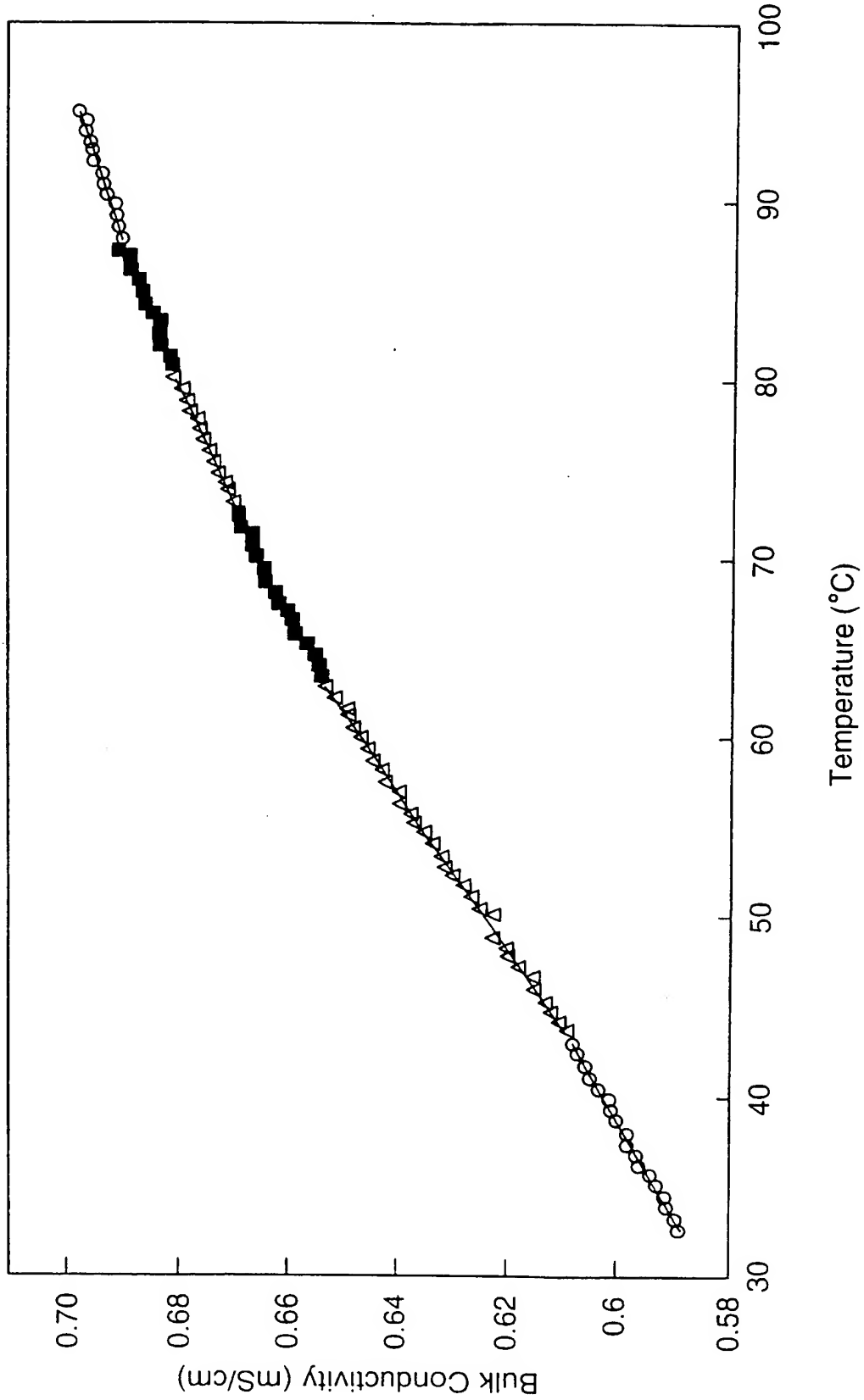


Fig.3.





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WO 01/59154 A3

(54) Title: PROCESS FOR CHARACTERISING NUCLEIC ACIDS IN SOLUTION

(57) Abstract: The invention provides a method for ascertaining information relating to nucleic acid material in a solution, comprising: measuring a parameter relating to the conductivity of the solution at a multiplicity of temperatures; analysing the measurements of said parameter; and detecting a discontinuity in the change of said parameter with temperature.

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/GB 01/00492

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 G01N27/02 G01N33/487

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N G06F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	WO 97 32039 A (HYBAID LTD) 4 September 1997 (1997-09-04) cited in the application the whole document	1-27

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 August 2001	07/09/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Hagenmaier, S

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	BONE STEPHEN ET AL: "Dielectric studies of ion fluctuation and chain bending in native DNA." BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1260, no. 1, 1995, pages 85-93, XP001016207 ISSN: 0006-3002 cited in the application the whole document	1-27
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